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Article

The Sensory Circuitry for Sexual Attraction in *C. elegans* Males

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Summary

Background: Why do males and females behave differently? Sexually dimorphic behaviors could arise from sex-specific neurons or by the modification of circuits present in both sexes. *C. elegans* males exhibit different behaviors than hermaphrodites. Although there is a single class of sex-specific sensory neurons in the head of males, most of their neurons are part of a core nervous system also present in hermaphrodites. Are the behavioral differences due to sex-specific or core neurons?
Results: We demonstrate that *C. elegans* males chemotax to a source of hermaphrodite pheromones. This sexual-attraction behavior depends on a TRPV (transient receptor potential vanilloid) channel encoded by the *osm-9*, *ocr-1*, and *ocr-2* genes. OSM-9 is required in three classes of sensory neurons: the AWA and AWC olfactory neurons and the male-specific CEM neurons. The absence of OSM-9 from any of these neurons impairs attraction, suggesting that their ensemble output elicits sexual attraction. Likewise, the ablation of any of these classes after sexual maturation impairs attraction behavior. If ablations are performed before sexual maturation, attraction is unimpaired, demonstrating that these neurons compensate for one another. Thus, males lacking sex-specific neurons are still attracted to pheromones, suggesting that core neurons are sexualized. Similarly, transgender nematodes—animals that appear morphologically to be hermaphrodites but have a masculinized core nervous system—are attracted to hermaphrodite pheromones.
Conclusions: Both sexually dimorphic and core sensory neurons are normally required in the adult for sexual attraction, but they can replace each other during sexual maturation if necessary to generate robust male-specific sexual attraction behavior.

Introduction

In the hermaphroditic nematode *C. elegans*, it is incumbent on the male to find a mate. There is considerable evidence for male-specific sexual attraction in nematodes (reviewed in [1–3]). *C. elegans* males remain on a food source if a hermaphrodite is present [4] and respond to cues released specifically by hermaphrodites [5]–clear evidence for male-specific chemosensory behavior. What are the differences between the male and hermaphrodite nervous systems that could account for sexually dimorphic behaviors? At first glance, the nervous systems of hermaphrodites and males are radically different. Out of 383 total neurons in males, 89 are sex-specific. However, most of the sex-specific neurons in the male innervate the specialized copulatory apparatus of the male, the male tail. Thus, much of the male-specific circuitry is likely to be involved in copulation rather than chemotaxis to hermaphrodites [6]. The remaining 294 neurons in the male comprise a core nervous system also found in hermaphrodites [7]. All of the sensory neurons in the head, responsible for chemotaxis of hermaphrodites to simple compounds, are also found in males. Consistent with this anatomical similarity, the chemosensory responses of the two sexes to simple compounds are to a first approximation the same [8]. In addition, males have four sex-specific chemosensory neurons in the head, the CEM neurons. These neurons undergo programmed cell death during hermaphrodite development, surviving only in males [7]. The male-specific CEMs have long been hypothesized to mediate chemosensory mate finding behavior in *C. elegans* [9]. Thus, male-specific sexual attraction could be due solely to the sex-specific CEM neurons, to subtle differences in the core circuitry, or a combination of the two.

Results

Male Sexual-Attraction Behavior

C. elegans males tax to a source of hermaphrodite pheromones and tend to remain there (Figures 1 and 2A). To characterize this response in *C. elegans*, we adapted a two-spot assay similar to those developed for studies of chemotaxis [10–12]. This assay demonstrates that *C. elegans* males tax to the peak of a pheromone gradient and remain at the source (Figures 1 and 2A). To distinguish the chemosensory behaviors elicited by pheromones from behaviors requiring tactile input, we used media conditioned by hermaphrodites (see the Supplemental Experimental Procedures and Figure S2 in the Supplemental Data available online). To generate conditioned media, we grew *C. elegans* hermaphrodites in liquid culture, removed the worms and bacteria food, and filtered the remaining liquid (Supplemental Experimental Procedures). Drops of conditioned and unconditioned media were placed on an agar test plate and allowed to soak in, and a thin coat of bacterial food was spread over the entire surface. Males on a test plate locate the source of hermaphrodite-conditioned media from a distance and tend to stay there (Figures 1, 2A, and 2B). Males respond to pheromones at a distance of up to 30 mm; analysis of single males (Figures 2C and 2D, Figure S1) shows that once an individual starts, he continuously moves toward the source until he is within 10 mm of the source center (Figure 2D, Figure S1). Attraction is specific to adult males; male L4 larvae and hermaphrodites do not

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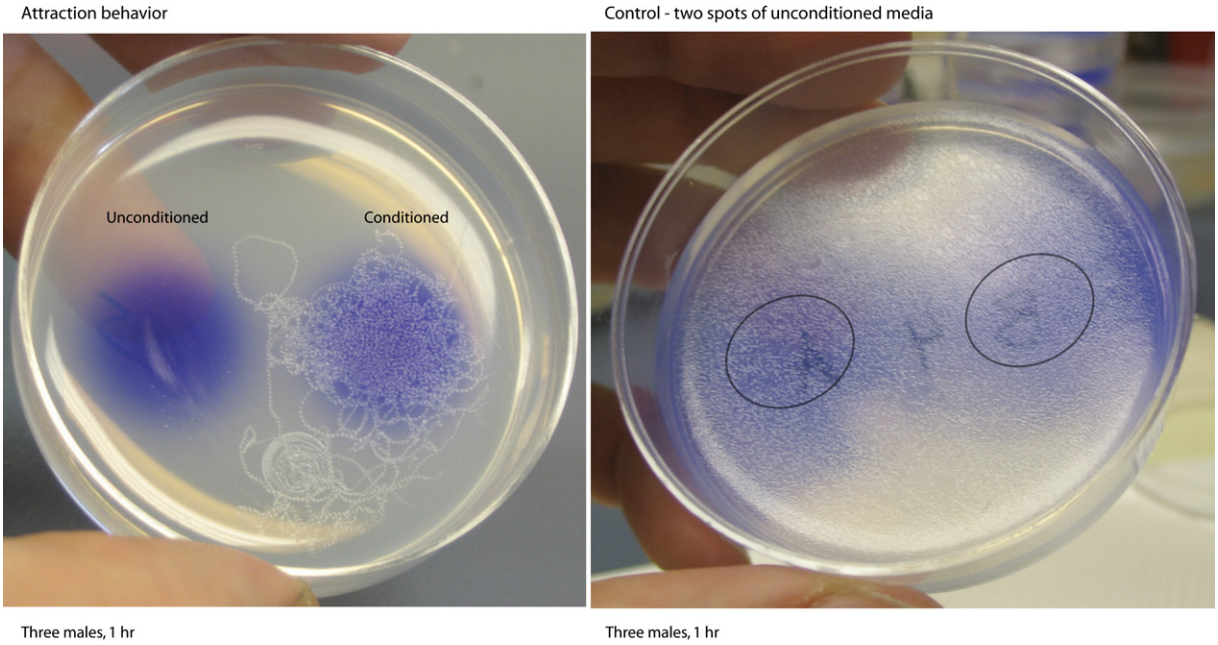

Figure 1. Male Sexual-Attraction Behavior

Males tax to a spot of hermaphrodite-conditioned media on an agar plate and tend to stay there once they have found it. Shown are photos of two-spot assays with three males each. Assays are on 50 mm (as measured) nematode growth media (NGM) agar plates spread with a thin layer of bacterial food 30 min prior to the start of the assays. Bromophenol blue to a final concentration of 0.1% (w/v) is added so that the spot on the plate could be visualized. The left panel is an assay with a control spot and a spot of hermaphrodite-conditioned media. The right panel is a control assay with two unconditioned spots. We score visual assays by the tracks males leave in the food, visible over the spot of conditioned media in the left panel. The control plate has many tracks over the entire surface of the plate. In blind assays, the plate on the left would be scored as positive for attraction, and the plate on the right would be negative.

have detectable attraction behavior (Figure 2E). Because only sexually mature males tax to hermaphrodite pheromones, and because it allows them to find their mating partners, we refer to this behavior as male sexual attraction (or simply male attraction).

Sexual Attraction Requires the TRPV Channel OSM-9

Male sexual attraction appears similar to known chemotaxis behaviors (Figure 2, Figure S1, [11, 12]). Chemosensation in *C. elegans* is mediated by ciliated neurons [13]. The proper development of ciliated sensory neurons requires the intraflagellar transport complex genes *osm-5* and *osm-6* [14, 15], and a subset—those exposed to the external environment—require a kinesin encoded by *osm-3* [14, 15]. *osm-5* and *osm-6* mutant males are partially defective for attraction (Figure 3A), so attraction is mediated by ciliated sensory neurons. The incomplete effect might be due to the potency of the attraction signal because *osm-5* and *osm-6* are only partly defective for chemotaxis behaviors to some volatile odorants [16]. *osm-3* mutants respond to volatile odorants but fail to respond to water-soluble attractants [11]. *osm-3* mutant males have no detectable defect in attraction behavior, suggesting that the attraction pheromone might be more similar to volatile odorants than to soluble signals.

In general, chemosensory behaviors in *C. elegans* require either a particular TRPV (transient receptor potential vanilloid) channel, containing OSM-9, or a particular cyclic-nucleotide-gated channel, containing TAX-2 [17]. Each channel is required for multiple, mostly nonoverlapping sets of sensory behaviors—OSM-9 for olfaction, osmosensation, and nose touch, and TAX-2 for gustation, thermosensation, and a different set of olfactory behaviors [17]. The OSM-9 TRPV channel might include additional subunits [18], and TAX-2 forms a cyclic guanosine monophosphate (cGMP)-gated channel with TAX-4 [19, 20]. To place male attraction into one of these broad sensory classes, we tested mutants in the TAX-2 and OSM-9 pathways (Figure 3B). *tax-2* and *tax-4* mutants have wild-type attraction behavior. Consistent with this, mutants in *daf-11,* which encodes a guanylate cyclase [21], also exhibit wild-type attraction. In contrast, *osm-9* mutants are defective for attraction (Figures 3B and 3C).

The defect in *osm-9* mutants is strong (Figure 3B) but often not complete (for example, *ky10* in Figure 3C), possibly indicating that OSM-9 is redundant with another TRP channel. We therefore tested mutants in the closely related channels OCR-1 and OCR-2, which are coexpressed in many of the same cells as OSM-9 [18]. The *ocr-1* and *ocr-2* single mutants have wild-type attraction behavior (Figure 3C). However, the *ocr-2*; *ocr-1* double mutant has an impaired attraction response similar to *osm-9*, but the triple *osm-9 ocr-2*; *ocr-1* mutant is no worse than *osm-9* (Figure 3C). The simplest genetic interpretation of these results is that *ocr-1* and *ocr-2* are redundant with one another and function in the same pathway as *osm-9*, consistent with the idea that OCR-1 and OCR-2 can each function as heteromer with OSM-9 [18]. Additionally, we tested mutants in the male-exclusive TRPP (transient receptor potential polycystin) channels encoded by *lov-1* and *pkd-2* (Figure 3D). *lov-1*

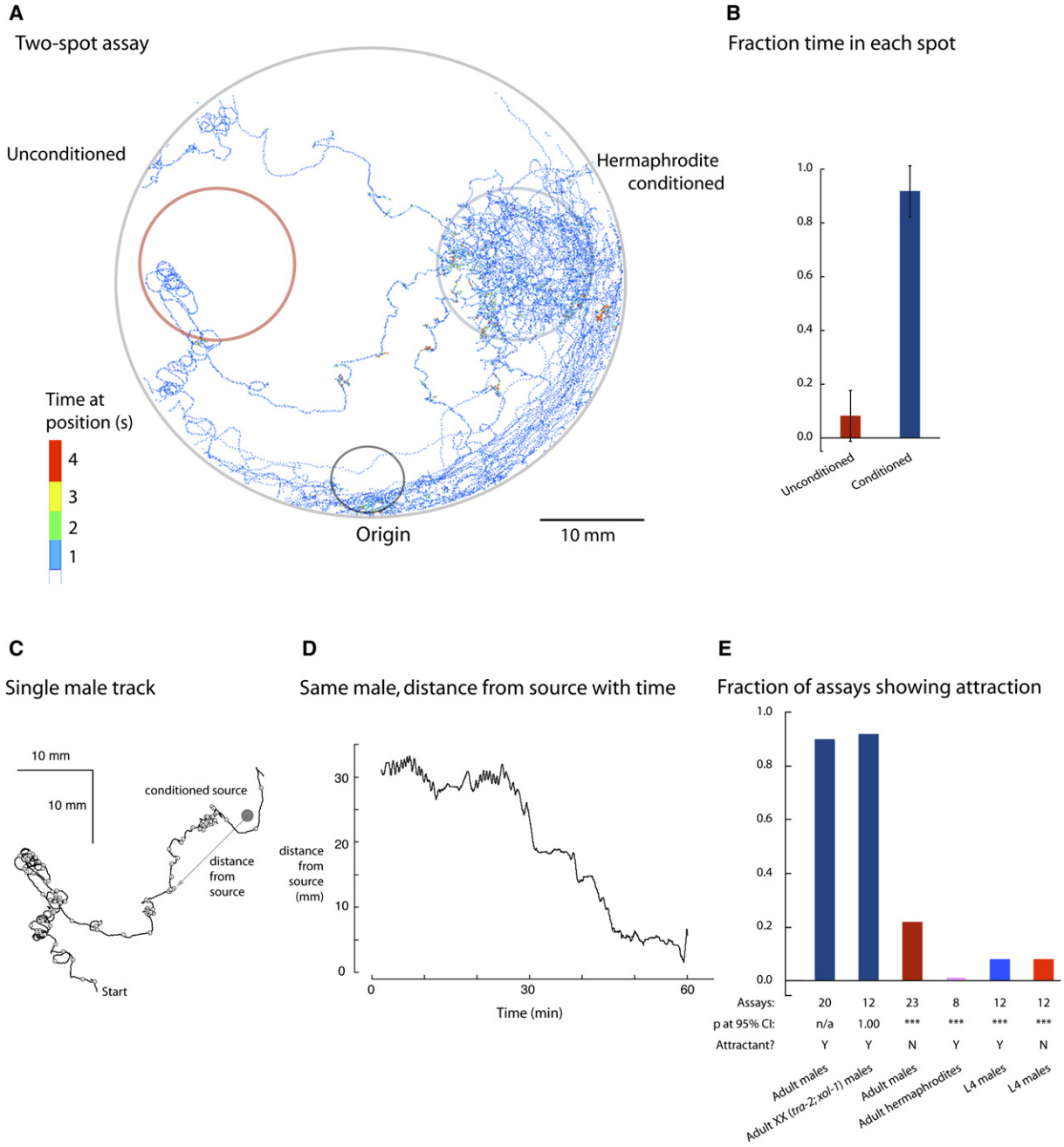

Figure 2. Sexual Attraction Is a Long-Range Chemotaxis Behavior Specific to Adult Males

(A) Video tracking of males while they are finding a spot of hermaphrodite-conditioned media. We recorded videos of ten males on a plate at 1 frame/second for 60 min and analyzed them by using computer tracking. The cumulative positions of all ten males over the entire hour are shown for one experiment, along with the boundary of the plate, the positions of the unconditioned- and conditioned-media spots, and the origin from which the males were released. A warmer pixel color indicates that worms spent a longer time at a given position.

(B) Males prefer hermaphrodite-conditioned spots. We analyzed the time males spent in either the conditioned or unconditioned spot in three independent tracking experiments. The plot shows the time the males spent in the one spot or the other normalized to the total time spent in either spot. Error bars show the 95% confidence interval of the average time for three independent tracking experiments.

(C) The track of a single male from the analysis in (A). The male suppresses looping and backing and tends to make continuous forward runs until he is within 5 mm of the center of the conditioned spot. Circles are at positions 30 s apart. Additional examples are in Figure S1.

(D) Attraction is elicited at a distance. The distance of the male in (C) from the center of the conditioned spot as a function of time is shown. The male remains at an approximately 30 mm away until 25 min into the assay, and then continuously moves toward the spot until he is within 5 mm, at about 45 min into the assay. He remains approximately 5 mm away from the center until the assay ends. Additional examples are in Figure S1.

(E) Attraction behavior is specific to adult males. The attraction responses were scored visually, blind for both the identity of animals on the plate and the identity of the spot (conditioned or unconditioned). Males tend to commit suicide by crawling off the side of the plate, so we use three males in visual assays to increase reliability. At least two assays were performed per condition on three different days, and the results combined and normalized for comparison. The total number of assays for each condition is indicated below each bar. Fisher's exact test with the Bonferroni-Holm correction for multiple comparisons was used to compare each response to wild-type ("p at 95% CI" [CI: confidence interval]); "***" indicates $p < 0.0001$.

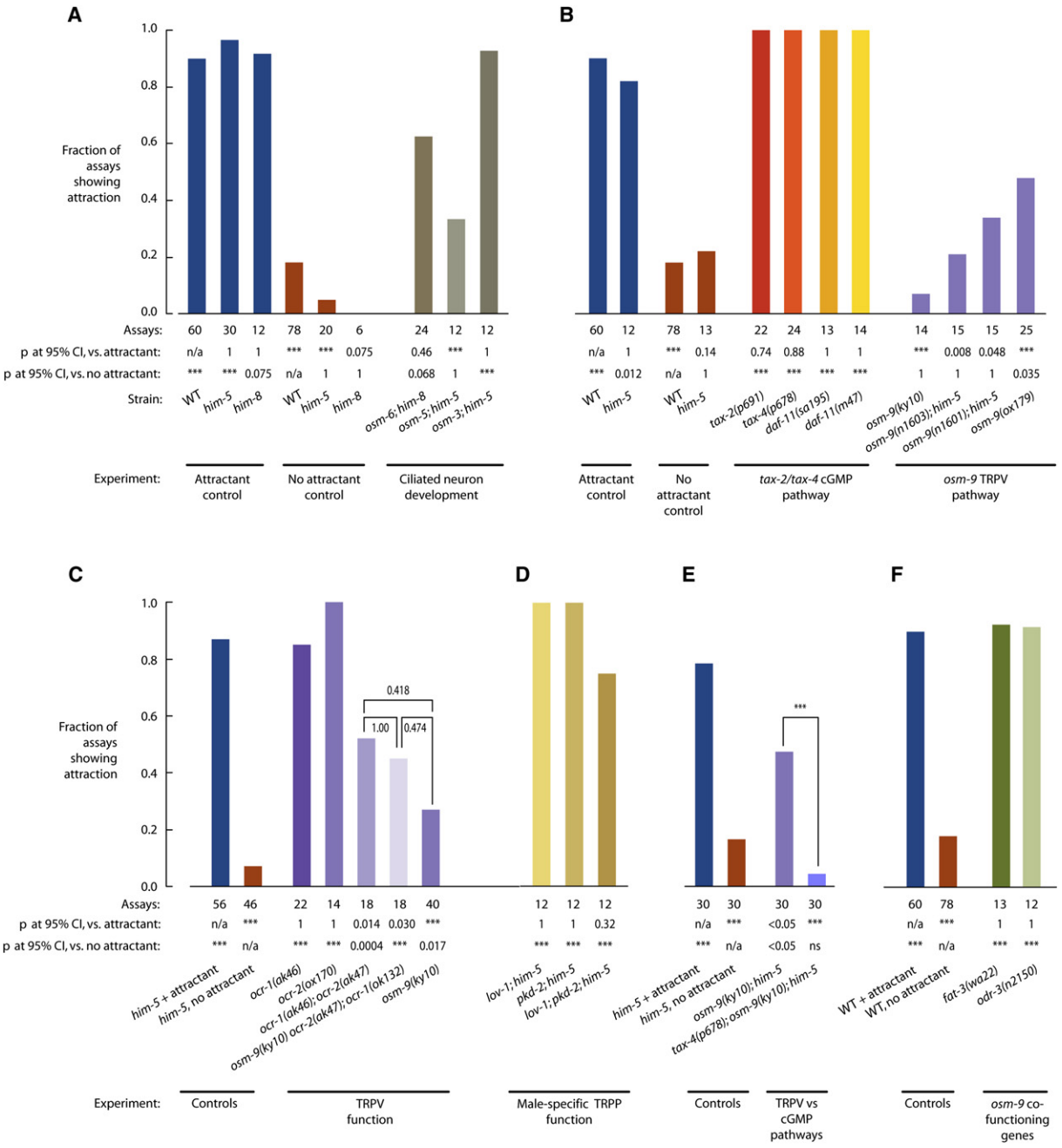

Figure 3. Male Attraction Behavior Requires a *TRPV*-Dependent Sensory Signaling Pathway, with Contributions from cGMP-Dependent Sensory Signaling

For each condition, bars represent the fraction of the indicated number of assays showing attraction behavior, scored blind, performed on at least three separate days. For each set of experiments, Fisher's exact test was used to compare the response of each strain to its corresponding *him-5*, *him-8*, or N2 Bristol conditioned media control ("p at 95% CI, attractant") and its unconditioned media control ("p at 95% CI, no attractant"). The Bonferroni-Holm correction was applied for multiple comparisons. "****" indicates $p < 0.0001$.

(A) Attraction behavior requires genes specific for ciliated sensory neuron development. The genotypes analyzed were N2 Bristol, *him-8(e1489)*, *him-5(e1490)*, *him-8(e1489) IV; osm-6(p811) V*, *him-5(e1490) V; osm-5(p813)*, and *osm-3(p802) IV*.

(B) Attraction behavior is impaired in *osm-9* mutant males, but not in single mutant males defective for cGMP signaling mediated by *tax-2* and *tax-4*.

(C) Attraction requires *ocr-1* and *ocr-2*, which are redundant with one another and act in the same pathway as *osm-9*. Fisher's exact test with Bonferroni-Holm correction for multiple comparisons was used for pairwise comparisons between the *ocr-2 osm-9*; *ocr-1* triple mutant, the *ocr-2*; *ocr-1* double mutant, and the *osm-9* single mutant.

(D) Attraction behavior does not require the male-specific TRPP channel subunits encoded by *lov-1* or *pkd-2*. Genotypes analyzed were *him-5(e1490)*, *lov-1(sy582); him-5(e1490), pkd-2(sy606); him-5(e1490),* and *lov-1(sy582); pkd-2(sy606); him-5(e1490)*.

(E) TAX-4-dependent cGMP signaling contributes to attraction behavior in the absence of OSM-9. Fisher's exact test with the Bonferroni-Holm correction was used to compare the *osm-9(ky10)* single mutant and the *tax-4(p674); osm-9(ky10)* double mutant.

(F) Genes that often function in *osm-9* pathways are not required for attraction. *fat-3* encodes an omega-3 lipid desaturase required for the synthesis of polyunsaturated fatty acids, which in some cases are required for function of OSM-9. *odr-3* encodes a G_{α} subunit that in some cases is thought to activate OSM-9.

and *pkd-2* are expressed in specialized sensory neurons not present in hermaphrodites, including the CEM neurons, the hook neuron HOB, and the sensory ray neurons, and are required for mechanosensory steps of male mating [22, 23]. It might be expected that these genes are required for other male-specific sensory behaviors, such as attraction. However, *lov-1* single-, *pkd-2* single-, and *lov-1; pkd-2* double-mutant males have unimpaired attraction behavior (Figure 3D). This is perhaps not surprising, given evidence leading to the hypothesis that TRPP channels directly sense movement or mechanical stimuli [24] rather than function in chemosensation. Because OSM-9- and TAX-4-containing channels are in some cases required for different functions in the same neuron (AWC, for example [17]), we tested the behavior of *osm-9; tax-4* double-mutant males (Figure 3E). Although a *tax-4* mutation has no effect on its own, it eliminates the remaining attraction behavior of an *osm-9* mutant ($p < 0.001$, Figure 3E). Thus, TAX-4 cGMP signaling is not absolutely required for male sexual attraction, but it might act in some neurons to assist OSM-9 signaling, and it accounts for the remaining behavior in an *osm-9* mutant.

Mutants with defects in genes that typically function with the OSM-9 TRPV channel have normal attraction behavior (Figure 3F). In nematodes, OSM-9 TRPV channels are thought in some circumstances to be activated by G protein signaling [24]. The *C. elegans* G_{α} subunit encoded by *odr-3* is expressed in the *osm-9* olfactory neurons AWA and AWC and has phenotypes similar to *osm-9* [25]. However, *odr-3* mutant males have normal attraction behavior. Sensory G proteins might activate OSM-9 by mobilizing specific polyunsaturated fatty acids (PUFAs), the synthesis of which depend on an omega-3 lipid desaturase encoded by the *fat-3* gene [26]. *fat-3* mutants have sensory phenotypes similar to *osm-9* and can be rescued by the exogenous application of PUFAs in an *osm-9*-dependent manner [26]. However, *fat-3* mutant males have normal attraction behavior (Figure 3F). We speculate that there might be additional cofunctioning genes that we have not identified, or, more intriguingly, that OSM-9 might have a unique function in male attraction.

Sexual Attraction Requires Both Male-Specific and Core Sensory Neurons

To determine the sensory neurons required for male attraction, we performed laser-ablation experiments. Likely candidates to test by laser-ablation were neurons that express *osm-9*. In hermaphrodites, *osm-9* is expressed in the neuron pairs AWA, AWC, ASE, ADF, ADL, ASG, ASH, ASI, ASJ, ASK, IL2, OLQ, PVD, PHA, and PHB [17]. In males, by using the canonical *osm-9* transcriptional fusion (*osm-9::gfp5*, [17]), we see additional *osm-9* expression in male-specific neurons in the tail, possibly the RnB and HoB neurons, and in the male-specific CEM neurons in the head (data not shown, schematic in Figure 4A). It has long been hypothesized that the CEMs are sensory neurons that mediate male attraction to hermaphrodites [9]. Accordingly, we ablated the two pairs of CEM neurons in L4 males (Figure 4B) and assayed the operated animals as adults in single-animal, numerically scored attraction assays in parallel with mock-ablated controls. Attraction is greatly impaired in males with all four CEM neurons ablated. Males with one or more CEM neurons exhibit attraction behavior, although the response might be reduced (not shown). This indicates that the male-specific CEM neurons are required for sexual-attraction behavior.

Attraction has characteristics of a long-range chemosensory behavior (Figures 2C and 2D, [10–12]), implicating olfactory or gustatory neurons in the response. Of the *osm-9*-expressing neurons, AWA and AWC are major olfactory neurons, and ASE is a major gustatory neuron [10–12]. Notably, *osm-9* is required for all known sensory functions of AWA [24]. Accordingly, we ablated either the AWA or AWC pair in L4 males (Figure 4B). Attraction is greatly impaired in adult males lacking either AWA or AWC. In contrast, males in which the ASE pair is ablated have an attraction response that is not significantly different from mock controls (Figure 4B). Thus, in addition to the CEM neurons, both the AWA and AWC neurons, but not the ASE neurons, are required for male attraction behavior.

Male Sensory Neurons Can Compensate for One Another during Development

The ablation of the CEM, AWA, or AWC neurons impairs attraction behavior only when we perform the ablations on L4 larvae. The ablation of these neurons one set at a time in L3 larvae, earlier in development, did not detectably impair male attraction (Figure 4C). This is not simply due to a longer recovery period, because L4-ablated animals given the same amount of time to recover as L3-ablated animals (2 days) remain defective for attraction (data not shown). On this basis, we hypothesized that the male nervous system can compensate for the loss of the CEM, AWA, or AWC neurons if they are absent in the L3 stage or earlier. To test this hypothesis, we simultaneously ablated the CEM, AWA, and AWC neurons in L3 males. Adult animals with all three sets of neurons ablated at the L3 stage have impaired attraction behavior (Figure 4C). The attraction response of these animals is not significantly different than the single-set L4-ablated animals ($p > 0.05$, one-way analysis of variance [ANOVA] with Dunnett's multiple comparison test). Thus, in L3-ablated males, the CEM, AWA, and AWC neurons compensate for one another; the remaining sets of neurons adjust for the ablated set. In L4-ablated males, the nervous system no longer has this capacity.

Genetic mutants in which the CEM, AWA, or AWC neurons have lost their identity or are lost altogether should be equivalent to an ablation very early in development, and so should mimic the L3 ablation of these neurons. Accordingly, we tested strains with mutations that specifically eliminate the CEM neurons (*ceh-30,* Barbara Conradt and Phillip Grote, personal communication), the AWA neurons (*odr-7* [27]), or the AWC neurons (*ceh-36* [28, 29]). Consistent with our L3 ablation results, single and double mutants had no significant effect on male sexual-attraction performance (Figures 4D and 4E), but the *ceh-30 odr-7 ceh-36* triple mutant has strongly impaired male attraction behavior (Figure 4F). Notably, the ASE neurons are always present in the triple mutant, indicating that they are not required for attraction, and so contribute very weakly, if at all. Thus, the genetic removal of the CEM, AWA, and AWC neurons

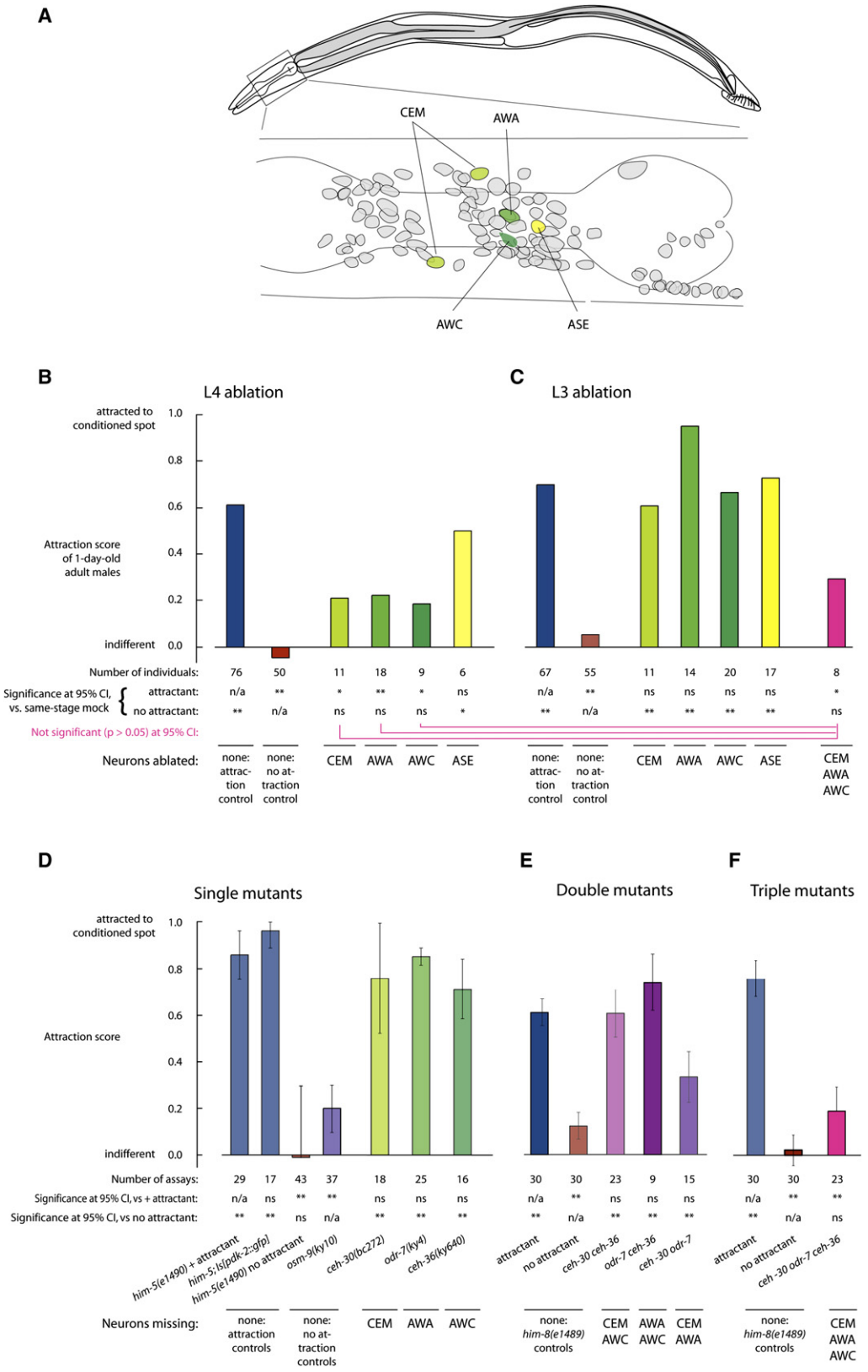

Figure 4. Male Sexual Attraction Requires the AWA and AWC Olfactory Neurons and the Male-Specific CEM Neurons, All of which Can Compensate for One Another

(A) Schematic of male neuroanatomy. A diagram of a *C. elegans* male with the head ganglia shown enlarged, indicating the anatomical position of the CEM, AWA, AWC, and ASE neuron nuclei. Only the left side is shown; bilaterally symmetric neurons are found on the right side. Diagrams are adapted from [33, 36].

recapitulates the removal of these neurons by laser ablation, demonstrating that they are the main sensory neurons required for male attraction behavior and supporting the conclusion that they compensate for one another if removed early in development.

Sexual-Attraction Behavior Depends on the Ensemble Output of the AWA, AWC, and CEM Neurons

To determine whether additional *osm-9*-expressing neurons might contribute to male sexual attraction, we rescued *osm-9* in combinations of the AWA, AWC, and CEM neurons in an *osm-9(ky10)* mutant background by using cell-specific promoters (Figure 5), and we assayed the attraction behavior of the rescued strains. The expression of *osm-9* in each single neuron class and in the CEM + AWC combination did not restore attraction behavior; these males were not significantly different from the *osm-9(ky10)* control. Expression in the CEM + AWA and AWA + AWC combinations resulted in attraction behavior that was not significantly different either from the *osm-9(ky10)* or from *him-5* controls, suggesting intermediate behavioral performance. Concurrent expression in the CEM, AWA and AWC neurons completely restored behavioral performance, and was comparable to a strain in which *osm-9(+)* is expressed in the entire nervous system. The fully rescued males are not significantly different from the *him-5* control and are significantly different from the *osm-9(ky10)* control. These results indicate that fully functioning AWA, AWC, and CEM neurons are sufficient for male attraction behavior; if other *osm-9*-expressing neurons contribute, their contribution must be minor. Also, because the *pkd-2* promoter used to drive CEM expression in these experiments only comes on strongly in the adult [22, 23], OSM-9 must function in—or maintain the function of—the adult circuit, and so is most likely not required for the developmental wiring of the male sexual-attraction sensory circuit. Finally, these results indicate that AWA, AWC, and CEM function is nonredundant—it is necessary for all three classes to be active to achieve full behavioral rescue, consistent with the L4 ablation results.

Reversing Sexual Preference

Only males are attracted to hermaphrodite pheromones. Hermaphrodites could in principle be attracted to pheromones, but they are not. What is the site of the sex specificity of male attraction behavior? We considered two possibilities: One is that sex-specific hormonal signals from the gonad or other nonneuronal tissues influence the function of the core nervous system, and the other is that sexual attraction is intrinsic to the male circuitry and so depends only on the sex of the nervous system. To distinguish these possibilities, we masculinized the nervous system of hermaphrodites. The overexpression of *fem-3* throughout an entire XX animal during development is sufficient to masculinize the animal and turn off hermaphrodite-specific genes [30]. We therefore expressed the *fem-3* complementary DNA (cDNA) from the nervous-system-specific *rab-3* promoter to generate animals that were hermaphrodites with respect to karyotype and overt morphology but expressed *fem-3* at high levels in the nervous system. The overexpression of *fem-3* in the nervous system was sufficient to induce survival of the male-specific CEM neurons in ~1% of animals, revealed by a P(*pkd-2)::gfp* reporter (data not shown). Male-specific neurons in the tail were never observed because the progenitors of these cells differentiate as skin cells in hermaphrodites rather than neurons as in males [31–33] and never express neuronal genes such as *rab-3*. Hermaphrodites with masculinized nervous systems have robust attraction behavior that is indistinguishable from control males (Figure 6) and significantly different from nonmasculinized hermaphrodites. This result demonstrates that the sex-specificity of male attraction behavior is determined solely by the sexual identity of the nervous system. Masculinization of the core neurons is sufficient for attraction to hermaphrodites.

Discussion

Why are males attracted to hermaphrodite pheromones, but hermaphrodites not? Our results demonstrate that the sex specificity of male attraction depends only on

(B) L4 ablation of either the AWA, AWC, or CEM neurons severely impairs attraction behavior in adult males. Bars represent the average score of all ablations at each condition. One-way ANOVA with Dunnett's multiple comparison test was used to compare ablated males to control animals tested with attractant or no attractant ("Significance at 95% CI, vs. mock"); "**" indicates $p < 0.01$, "*" indicates $p < 0.05$, and "ns" indicates $p > 0.05$ (not significant).

(C) L3 ablation of any single class of the AWA, AWC, or CEM neurons has no affect, but the simultaneous ablation of all three classes severely impairs attraction in adult males. Data representation and statistics are as in (B). Additionally, Dunnett's multiple comparison test was used to compare the CEM, AWA, AWC multiple L3 ablation to the L4 single ablations in (B) ($p > 0.05$).

(D) Attraction behavior of single mutant males deficient in the AWA, AWC, or CEM neurons is unimpaired. The following strains were tested: *him-5(e1490)*, MD549 *him-5(e1490) V; bcIs9[pkd-2::gfp] lin-15(n765ts) X*, MD1400 *him-5(e1490) V; bcIs9 ceh-30(bc272) X,* CX5922 *ceh-36(ky640) X ; kyIs140[str-2::gfp] I*, and CX4 *odr-7(ky4)*. Bars represent the average of three experiments on different days, and error bars indicate the standard error of the mean (SEM). One-way ANOVA with Dunnett's multiple comparison test was used to compare each strain to *him-5(e1490)* tested with attractant ("significance at 95% CI, vs. *him-5* + attractant"), and to *osm-9(ky10)* ("significance at 95% CI, vs. *osm-9(ky10)*"). "**" indicates $p < 0.01$, "*" indicates $p < 0.05$, and "ns" indicates $p > 0.05$ (not significant).

(E) Attraction behavior of double-mutant males in which pairs of the AWA, AWC, and CEM neuron class are affected is unimpaired. The following strains were tested: *him-8(e1489)*, EG4703 *him-8(e1489) IV ; bcIs9[P(pkd-2)::gfp] X ceh-30(bc272) X ceh-36(ky640) X ; oxEx1025[P(pkd-2)::gfp, P(ceh-36)::gfp, P(unc-17)::mCherry]*, EG4704 *him-8(e1489) IV ; odr-7(ky4) X ceh-36(ky640) X ; oxEx1028[P(odr-10)::gfp, P(ceh-36)::gfp, P(unc-17)::mCherry],* and EG4709 *him-8(e1489) IV ; bcIs9[P(pkd-2)::gfp] X ceh-30(bc272) X odr-7(ky4) X; oxEx1037[P(pkd-2)::gfp, P(odr-10)::gfp, P(unc-17)::mCherry].* Data representation and statistics are as in (D).

(F) Attraction behavior of triple-mutant males in which the AWA, AWC, and CEM neurons are simultaneously affected is severely impaired. We tested the sexual attraction behavior of *ceh-30(bc272) odr-7(ky4) ceh-36(ky640)* triple-mutant males in three sets of experiments on different days in three independently isolated mutant lines. We verified that the neurons predicted to be affected were indeed absent by checking for the absence of green fluorescent protein (GFP) expression from reporters specific to each cell. The genotype tested was *bcIs9[P(pkd-2)::gfp] X ceh-30(bc272) X odr-7(ky4) X ceh-36(ky640) X ; oxEx1023[P(pkd-2)::gfp, P(odr-10)::gfp, P(ceh-36)::gfp, P(unc-17)::mCherry]*. Data representation and statistics are as in (D).

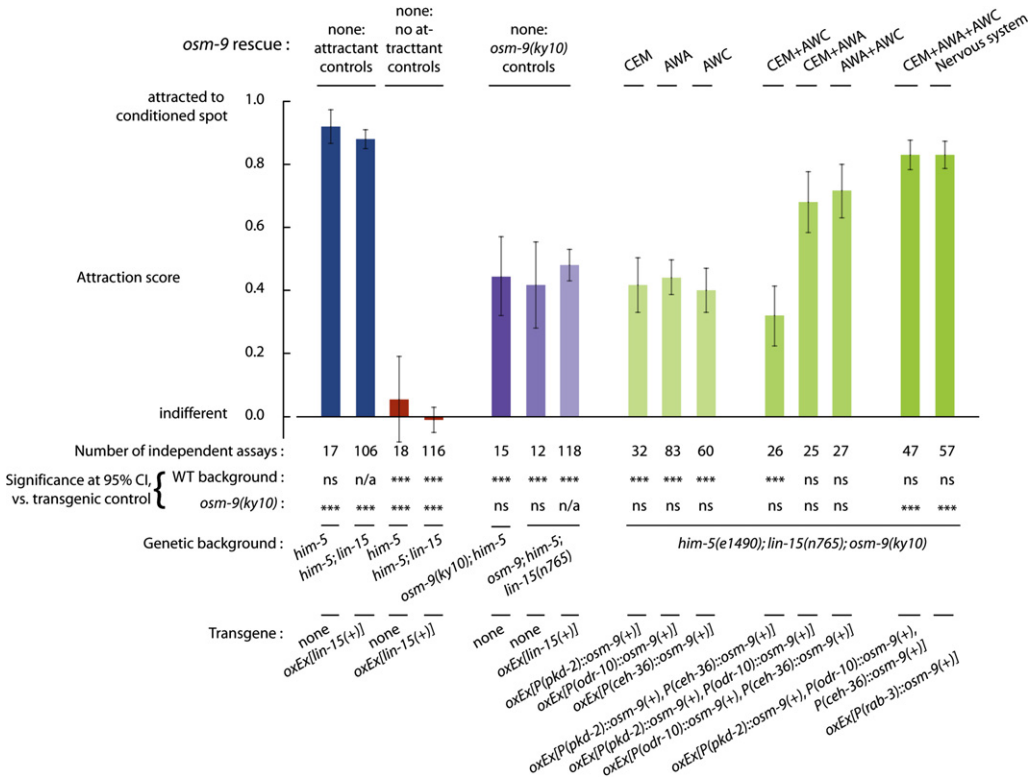

Figure 5. *osm-9* Rescue in the CEM, AWA, and AWC Neurons Completely Restores Attraction Behavior

We expressed a cDNA encoding the wild-type *osm-9* gene in the indicated combinations of the CEM, AWA, and AWC neurons in an *osm-9(ky10)* genetic background. Violet bars indicate *osm-9(ky10)* controls with no rescue. Light green bars show the behavior for expression in each single class, medium for pairs of classes, and dark green for all three classes or the entire nervous system. Bars represent the average of the total number of assays indicated; error bars indicate the SEM. One-way ANOVA with Dunnett's multiple comparison test was used to compare each strain to a *him-5(e1490)* transgenic control strain (EG4222 or EG4223, Table S1) tested with attractant ("significance at 95% CI, vs. transgenic control") and to *osm-9(ky10)* transgenic control strain (EG4244 or EG4255) tested with attractant ("significance at 95% CI, vs. *osm-9(ky10)*"). "***" indicates $p < 0.001$, and "ns" indicates $p > 0.05$ (not significant).

the sex of the nervous system, so the question further simplifies to the following: What are the differences between the male and hermaphrodite nervous systems that give rise to attraction behavior? Our results identify differences at both the molecular and neural levels.

At the molecular level, it is likely that there are sex-specific inputs into general OSM-9-dependent sensory signaling. Because OSM-9 is required for a number of sensory behaviors [17], the OSM-9/OCR-1/OCR-2 channel most likely mediates general sensory signal transduction downstream of a male-specific pheromone receptor or receptors in the adult sexual-attraction circuit. Our results indicate that the attraction defect in *osm-9* mutant males is due to a partially functioning sensory system rather than developmental differences within the population. The strong effect of *osm-9* mutations shows that OSM-9-dependent signaling is the major pathway, but because *osm-9* mutations do not completely abolish sexual attraction, there must be additional sensory signal-transduction pathways that contribute. One pathway appears to be a TAX-4-dependent cGMP signaling system because attraction behavior is completely abolished in a *tax-4*; *osm-9* double mutant. However, *tax-4* or *tax-2* mutants alone do not impair male attraction behavior, even at very dilute pheromone concentrations (J.Q.W. and T.J.N., unpublished data), so the relative contribution of a TAX-4 cGMP pathway in the presence of OSM-9 is unclear. It could perhaps have a sex-specific modulatory function on OSM-9-dependent signaling, or the converse. Regardless, it is likely that *osm-9* mutations affect male sexual attraction because they impair but do not abolish function of all of the sensory neurons required for attraction—AWA, AWC, and the CEMs.

Normally, the AWA, AWC, and CEM neurons are all required for male-specific sexual attraction, as shown by the L4 ablations. It appears as if attraction is not completely abolished in the ablated animals; however, their response is not significantly different from mock controls tested in assays without conditioned media, so the effect is strong, if not complete. This demonstrates that the AWA, AWC, and CEM classes of neurons are the major sensory neurons mediating male sexual attraction. In contrast to L4 ablation, the elimination of any single set of the AWA, AWC, or CEM neurons in L3 larvae or earlier, by either ablation or by mutation, does not impair sexual attraction. However, the concurrent removal of all three classes severely impairs attraction behavior, demonstrating that these are indeed the relevant sensory neurons. The male nervous system compensates for the early loss of neurons that are normally required in the adult.

Why does compensation not occur for the L4 ablations? The answer might have to do with the time during development when neurons are wired into a functional

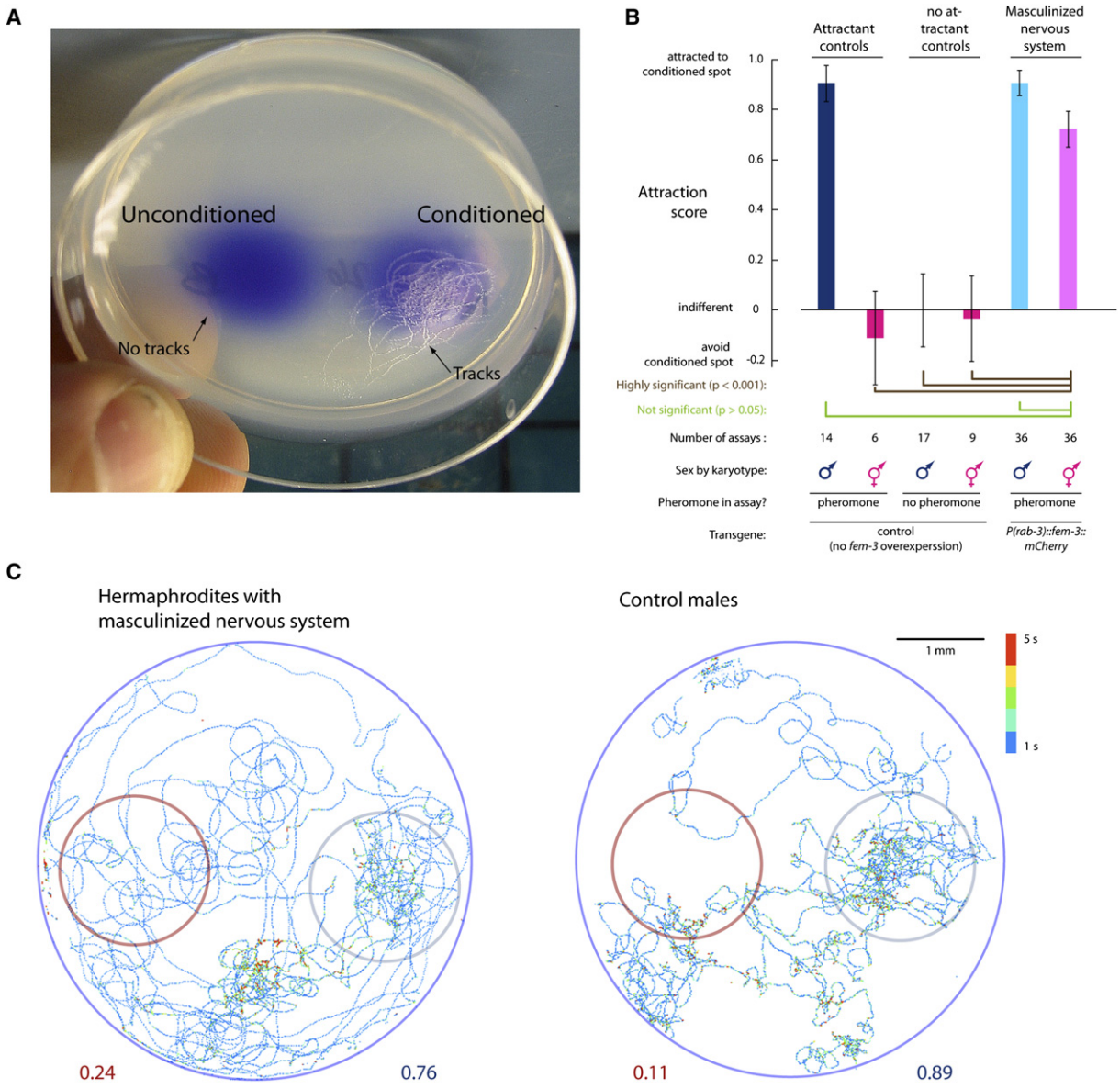

Figure 6. The Sex Specificity of Attraction Behavior Depends Solely on the Sexual Identity of the Nervous System

We masculinized the hermaphrodite nervous system by using the *rab-3* promoter to express a cDNA encoding the wild-type *fem-3* gene specifically in the nervous system. We assayed the attraction behavior of the transgenic XX hermaphrodites and, as a control, their XO male siblings.
(A) Attraction assay of three hermaphrodites with a masculinized nervous system. Reprogrammed hermaphrodites tax to the conditioned media spot and remain there, as shown by the tracks they leave on the plate. The elapsed assay time is 1 hr. The plate diameter is 50 mm.
(B) Attraction behavior in hermaphrodites with a masculinized nervous system is comparable to males. Attraction behavior was assessed in numerically scored, blind attraction assays. We assayed both XX and XO karyotypes in transgenic control strains (no *fem-3* expression; EG4222 or 4223) in the presence and absence of pheromone attractant. We compared these controls to both XO and XX individuals in strains expressing *fem-3* in the nervous system using the *rab-3* promoter. Bars represent the average of the total number of assays indicated; error bars indicate the SEM. One-way ANOVA with Bonferroni's multiple comparison correction was used to compare the response of each strain with all others.
(C) Video tracking of control males and reprogrammed hermaphrodites while they are finding a spot of hermaphrodite-conditioned media. We recorded videos of eight animals on a plate at 1 frame/second for 60 min and analyzed them by using computer tracking. The cumulative positions of eight hermaphrodites with a masculinized nervous system are shown on one plate and compared to the positions of eight sibling males on another. The boundary of the plates and the positions of the unconditioned- (red circle) and conditioned-media (blue circle) spots are indicated. A warmer pixel color indicates that animals spent a longer time at a given position. Numbers indicate the relative amount of time animals spent in the unconditioned (red) spot compared to the conditioned (blue) spot for each tracking experiment.

sensory circuit. During the L4 stage, the male nervous system undergoes dramatic remodeling to form the adult connections [31, 32]. It is likely that the attraction sensory circuit is wired at this time. L4 ablations could remove neurons from the circuit after the adult connections have been formed, or as they are forming. The male nervous system might not be able to compensate for the loss of a neuron after the final adult connections are formed. L3 ablations (and developmental mutations) could remove neurons before the adult connections have been formed. The remaining neurons somehow detect the absence and compensate for the missing

neurons during final wiring, perhaps by strengthening their connections to a common interneuron or by invading postsynaptic fields normally occupied by the missing neurons. Double mutants with only a single class of sensory neurons remaining still have robust attraction behavior, indicating that one set is sufficient. Similar strong compensatory effects allow the hypothalamic circuitry that normally promotes feeding in mice to be ablated in neonates with no affect on food intake [34]. Acute ablation in adults, however, causes mice to starve themselves [34, 35]. The neural circuitry that controls such fundamental behaviors such as sex and feeding seems to be particularly robust.

Losing a class of neurons is generally worse than losing their *osm-9*-dependent function. For example, males in which the CEM neurons have been ablated in L4 larvae have severely impaired attraction behavior, but *osm-9* mutant males in which *osm-9* is rescued in the AWA + AWC neurons have intermediate attraction behavior. One explanation is that an additional sensory signaling pathway in the CEM and AWC neurons—most likely a TAX-4-dependent pathway—contributes to the male attraction response. This is consistent with published results showing that TAX-4 is required for the primary olfactory functions of AWC [19, 20]. In one case, L4 ablation and rescue are equivalent: Males missing AWA by ablation and males missing *osm-9*-dependent AWA function (*osm-9* rescued only in the CEM + AWC combination) both have highly impaired attraction behavior. This suggests OSM-9 function in AWA is critically important for male attraction behavior, and it is consistent with the published observation that OSM-9 seems to be required for all known functions of AWA [24]. In all cases, *osm-9* rescue in a single class of neurons is not sufficient to fully restore male attraction behavior, indicating that the contributions of each class are to some degree nonredundant; inputs from each class must be combined to achieve full, robust behavior.

A male sensory system without the CEM neurons might be expected to be equivalent to that of a hermaphrodite, but it is not. To clarify, males lacking the CEM neurons, either by L3 ablation or by mutation [*ceh-30(bc272)*], have robust sexual-attraction behavior mediated by AWA and AWC. Hermaphrodites also lack CEM neurons and have AWA and AWC sensory neurons but do not have attraction behavior. Masculinizing the hermaphrodite nervous system demonstrates that the sole determinant of the sex specificity of sexual attraction behavior is the sex of the nervous system. Notably, most masculinized hermaphrodites lack CEM neurons but have robust attraction behavior. Therefore, the core *C. elegans* nervous system must be sexualized: Neurons common to both males and hermaphrodites are subtly modified to serve different sex-specific functions, giving rise to drastic differences in behavior.

Supplemental Data

Supplemental Results, Experimental Procedures, three figures, one table, and one movie are available at http://www.current-biology.com/cgi/content/full/17/21/1847/DC1/.

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References

1. Garcia, L.R., and Barr, M.M. (2006). Male mating behavior. WormBook, ed. The *C. elegans* Research Community, WormBook, doi:10.1895/wormbook.1.78.1, http://www.wormbook.org.
2. Emmons, S.W. (2006). Sexual behavior of the *Caenorhabditis elegans* male. Int. Rev. Neurobiol. *69*, 99–123.
3. Perry, R.N., and Aumann, J. (1998). Behaviour and sensory responses. In The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes, R.N. Perry and D.J. Wright, eds. (Wallingford, United Kingdom: CABI Publishing), pp. 75–92.
4. Lipton, J., Kleemann, G., Ghosh, R., Lints, R., and Emmons, S.W. (2004). Mate searching in *Caenorhabditis elegans*: A genetic model for sex drive in a simple invertebrate. J. Neurosci. *24*, 7427–7434.
5. Simon, J.M., and Sternberg, P.W. (2002). Evidence of a mate-finding cue in the hermaphrodite nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *99*, 1598–1603.
6. Liu, K.S., and Sternberg, P.W. (1995). Sensory regulation of male mating behavior in *Caenorhabditis elegans*. Neuron *14*, 79–89.
7. Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.
8. Dusenbery, D.B. (1976). Chemotactic responses of male *Caenorhabditis elegans*. J. Nematol. *8*, 352–355.
9. Ward, S., Thomson, N., White, J.G., and Brenner, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. *160*, 313–337.
10. Bargmann, C.I. (2006). Chemosensation in *C. elegans*. WormBook, ed. The *C. elegans* Research Community, WormBook, doi:10.1895/wormbook.1.123.1, http://www.wormbook.org.
11. Bargmann, C.I., Hartwieg, E., and Horvitz, H.R. (1993). Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell *74*, 515–527.
12. Bargmann, C.I., and Horvitz, H.R. (1991). Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. Neuron *7*, 729–742.
13. Inglis, P.N., Ou, G., Leroux, M.R., and Scholey, J.M. (2006). The sensory cilia of *Caenorhabditis elegans*. WormBook, ed. The *C. elegans* Research Community, WormBook, doi:10.1895/wormbook.1.126.1, http://www.wormbook.org.
14. Perkins, L.A., Hedgecock, E.M., Thomson, J.N., and Culotti, J.G. (1986). Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. *117*, 456–487.
15. Tabish, M., Siddiqui, Z.K., Nishikawa, K., and Siddiqui, S.S. (1995). Exclusive expression of *C. elegans osm-3* kinesin gene in chemosensory neurons open to the external environment. J. Mol. Biol. *247*, 377–389.
16. Hu, J., Bae, Y.K., Knobel, K.M., and Barr, M.M. (2006). Casein kinase II and calcineurin modulate TRPP function and ciliary localization. Mol. Biol. Cell *17*, 2200–2211.
17. Colbert, H.A., Smith, T.L., and Bargmann, C.I. (1997). OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. J. Neurosci. *17*, 8259–8269.

18. Tobin, D., Madsen, D., Kahn-Kirby, A., Peckol, E., Moulder, G., Barstead, R., Maricq, A., and Bargmann, C. (2002). Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in C. elegans neurons. Neuron *35*, 307–318.
19. Komatsu, H., Mori, I., Rhee, J.S., Akaike, N., and Ohshima, Y. (1996). Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. Neuron *17*, 707–718.
20. Coburn, C.M., and Bargmann, C.I. (1996). A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. Neuron *17*, 695–706.
21. Birnby, D.A., Link, E.M., Vowels, J.J., Tian, H., Colacurcio, P.L., and Thomas, J.H. (2000). A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. Genetics *155*, 85–104.
22. Barr, M.M., DeModena, J., Braun, D., Nguyen, C.Q., Hall, D.H., and Sternberg, P.W. (2001). The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs lov-1 and pkd-2 act in the same pathway. Curr. Biol. *11*, 1341–1346.
23. Barr, M.M., and Sternberg, P.W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. Nature *401*, 386–389.
24. Kahn-Kirby, A.H., and Bargmann, C.I. (2006). TRP channels in *C. elegans*. Annu. Rev. Physiol. *68*, 719–736.
25. Roayaie, K., Crump, J.G., Sagasti, A., and Bargmann, C.I. (1998). The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron *20*, 55–67.
26. Kahn-Kirby, A.H., Dantzker, J.L., Apicella, A.J., Schafer, W.R., Browse, J., Bargmann, C.I., and Watts, J.L. (2004). Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling in vivo. Cell *119*, 889–900.
27. Sengupta, P., Colbert, H.A., and Bargmann, C.I. (1994). The *C. elegans* gene *odr-7* encodes an olfactory-specific member of the nuclear receptor superfamily. Cell *79*, 971–980.
28. Koga, M., and Ohshima, Y. (2004). The *C. elegans ceh-36* gene encodes a putative homemodomain transcription factor involved in chemosensory functions of ASE and AWC neurons. J. Mol. Biol. *336*, 579–587.
29. Lanjuin, A., VanHoven, M.K., Bargmann, C.I., Thompson, J.K., and Sengupta, P. (2003). Otx/otd homeobox genes specify distinct sensory neuron identities in *C. elegans*. Dev. Cell *5*, 621–633.
30. Mehra, A., Gaudet, J., Heck, L., Kuwabara, P.E., and Spence, A.M. (1999). Negative regulation of male development in *Caenorhabditis elegans* by a protein-protein interaction between TRA-2A and FEM-3. Genes Dev. *13*, 1453–1463.
31. Sulston, J.E., Albertson, D.G., and Thomson, J.N. (1980). The *Caenorhabditis elegans* male: Postembryonic development of nongonadal structures. Dev. Biol. *78*, 542–576.
32. Emmons, S.W. (2005). Male development. WormBook, ed. The *C. elegans* Research Community, WormBook, doi:10.1895/wormbook.1.33.1, http://www.wormbook.org.
33. Sulston, J.E., and Horvitz, H.R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. *56*, 110–156.
34. Luquet, S., Perez, F.A., Hnasko, T.S., and Palmiter, R.D. (2005). NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science *310*, 683–685.
35. Ste Marie, L., Luquet, S., Cole, T.B., and Palmiter, R.D. (2005). Modulation of neuropeptide Y expression in adult mice does not affect feeding. Proc. Natl. Acad. Sci. USA *102*, 18632–18637.
36. White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. *314*, 1–340.